

IPD Project Details

Project ID: IPD8541

Project Title: Proteomic analysis of L-Methionine Sulfoximine (MSO)-treated *Plasmodium falciparum* by LC-MS/MS

Description: LC-MS/MS of in-solution trypsin digested parasite protein extracts was carried out to examine the proteome of MSO-treated Pf parasites. Proteins were extracted from the untreated and MSO-treated parasite pellets of two independent experiments (Exp1: T7_R Vs T8; Exp2: T3_R Vs T4_R). For each experiment, two different sets of untreated and MSO-treated cultures synchronized for ring stages and early trophozoites were used. MSO treatment was carried out at 50 μ M concentration for 12 h. A total number of 149 proteins associated with various metabolic and cellular functions, cytoadherence and host invasion, Hb degradation, etc., were downregulated in MSO-treated parasites. This also included important asparagine-rich proteins such as tRNA ligases, components of RNA processing and protein degradation pathways, lipocalin associated with hemozoin formation and antimalarial drug sensitivity, heat shock protein 110c essential for stabilizing the asparagine repeat-rich parasite proteins etc. Only ten proteins were found to be upregulated in MSO-treated Pf3D7 parasites. For downregulated proteins, proteins identified in both the untreated controls of two independent experiments and either undetectable or significantly downregulated (>1.5 fold) in MSO-treated Pf3D7 parasites were considered. For upregulated proteins, proteins significantly upregulated (>1.5 fold) in both the MSO-treated parasites of two independent experiments and/or undetectable in the untreated controls but detectable in the MSO-treated parasites were considered.

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Sample Preparation: The untreated and MSO-treated parasite pellets isolated by saponin treatment were pooled separately during solubilization with 6 M urea in 25 mM ammonium bicarbonate buffer. The solubilized protein samples were then treated with DTT and iodoacetamide, and the urea concentration was decreased to 0.6 M by diluting with 25 mM ammonium bicarbonate.

Peptide Separation: In-solution trypsin digestion was carried out overnight at 37°C for 300 μ g total protein using TPCK-treated trypsin (Sigma-Aldrich, 4352157). LC-MS/MS

was performed with micro flow reverse-phase LC in Eksigent Ekspert Nano LC 425 system (SCIEX) that was directly connected to a tandem quadrupole time-of-flight SCIEX TripleTOF 5600+ ESI-mass spectrometer. SCIEX Micro Trap Cartridge (Chrome XP; C18-CL, 5- μ m, 120-Å pore size) was used to concentrate the samples. 0.1% (v/v) formic acid and 2% (v/v) acetonitrile in water was used to wash the trap cartridge, followed by the separation of the concentrated peptides using a SCIEX capillary reverse-phase column (ChromeXP, 3C18-CL-120, 3 μ m, 120 Å and 0.3 $\times$ 150 mm). The flow rate was set at 5 μ l/min and the run was carried out using following solvents: solvent A - 98% water and 2% acetonitrile containing 0.1% formic acid (v/v), and solvent B - 98% acetonitrile and 2% water containing 0.1% formic acid (v/v). The gradient followed was 2% to 50% of solvent B for 28 min, followed by 50% to 90% of solvent B for 1 min, sustaining 90% of solvent B for 3 min and then 90% to 5% B for 0.5 min with a final re-equilibration with 2% of solvent B for 2.5 min. Mass spectra and tandem mass spectra were recorded in positive-ion and high-sensitivity mode with a full scan resolution of 35,000 (full width at half maximum). The following parameters were used for ion source: IonSpray Voltage Floating (ISVF) = 5500; Ion Source Gas 1 (GS1) = 25; Ion Source Gas 1 (GS2) = 22; Curtain Gas Flow (CUR) = 30. The precursor ions were fragmented with nitrogen gas in a collision cell. 100 fmol beta-galactosidase digest (SCIEX) was used to calibrate TOF MS and TOF MS/MS spectra. The peptide spectra were recorded over a mass/charge (m/z) range of 350 to 1250, and MS/MS spectra were recorded over an m/z range of 150 to 1600 in data-dependent acquisition (DDA) mode.

Protein Characterization: Analyst TF1.7.1. software was used for data acquisition and DDA was performed to obtain MS/MS spectra for the 15 most abundant parent ions following each survey MS1 scan (250-ms acquisition time per MS1 scan and 50-ms acquisition time per MS/MS). Dynamic exclusion features were set to an exclusion mass width of 50 mDa and an exclusion duration of 6 s. Paragon algorithm (ProteinPilot Software Version 5.0.2, SCIEX) was used to annotate MS/MS data against the reference proteomes of *Plasmodium falciparum* available at Uniprot (<https://www.uniprot.org/>) with the following parameters: TripleTOF 5600 instrument; alkylation of cysteines by iodoacetamide; trypsin enzyme digestion; ID Focus on biological modifications and the detected protein threshold [Conf] set at >10%. Peptides identified with ≥ 95 confidence were selected to calculate the fold change from the cumulative peptide intensities of the respective proteins. Cumulative peptide intensities of the identified proteins were normalized with respect to parasite actin I before determining Log2FC values.

Experiment Type: Top-down

Species: Data in species_details No Data

Tissue: Data in tissue_details No Data

Cell Type: Data in cell_details No Data

Disease: Data in disease_details No Data

Instrument Details: Data in instrument_details Data in instrument_details

Protein Modifications: iodoacetamide derivatized residue

PubMed ID: